Microbiology 581.02
Microbial Genetic Laboratory
Course Syllabus

Microbiology 581.02 Microbial Genetic Laboratory. (3 cr.)
Autumn, Winter and Spring quarters

Instructors:

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Laboratory schedule:

Two three-hour lab sessions per week; T R, 9:30-12:18 and M W, 1:30-4:18. Lab will be held in the Biological Sciences building, room 311.

Course Objectives:

This is a required laboratory course for Microbiology Majors and complements the required lecture course, Microbial Genetics, Micro 581.01. Micro 581.02 provides an introduction to experimental methods in the structure, maintenance, expression and exchange of genetic materials in microbial cells. The laboratory is presented as four modules: Mutagenesis of green fluorescent protein; Transposon mutagenesis and analysis of Lac– mutants; Transposon mutagenesis and analysis of auxotroph mutants; Transposon mutagenesis and analysis of motility mutants. Each module builds on concepts presented in the foundation lecture course and illustrates concepts in experimental design and data analyses. Students will gain experience in numerous techniques, including both wet-lab approaches and computational analysis of numerical and DNA/protein sequence data.

Students completing the course will:

1. Understand the basic principals of experimental design as it pertains to the production and analysis of mutations in microbial cells.
2. Develop an understanding of the theory of modern molecular biological techniques and acquire hands-on experience in their application. This will encompass a variety of methods including the isolation and analysis of nucleic acids, the introduction of foreign DNA into microbial cells and the analysis of mutant phenotypes, site specific and transposon-mediated mutagenesis, and the bioinformatic analyses of DNA and protein sequences.

3. Develop experience in the interpretation and presentation of experimental results as written module reports.
4. Develop an understanding of how experimentation has defined our current understanding of microbial systems and how modern approaches are applied in analysis of new problems.

Text:
Microbiology 581.02 Lab Manual: Drs. Kathleen Sandman, Brian Ahmer and Irina Artsimovitch. 2008. A number of primary literature references are cited in the manual and serve as additional readings.

The course materials (data sets and student results), laboratory quizzes, study guides and review materials are also presented on the Carmen site for the course.

Grading:

Quiz	20 points
Final lab exam	20 points
Safety quiz	3 points
15 open book quizzes (Carmen) @3 points each	45 points
6 homework assignments @ 7 points each	42 points
3 module reports @ 20 points each	60 points
Laboratory Skills	5 points
Attendance	5 points
	200 points

Academic misconduct:

Academic misconduct will not be tolerated and will be dealt with as defined in the Code of Student Conduct: http://studentaffairs.osu.edu/resource_csc.asp and http://trustees.osu.edu/Rules%2023/index.php.

Disability Services:

Any student who may need an accommodation because of a disability should contact the instructor privately to discuss specific needs. The Office for Disability Services assists faculty in verifying the need for accommodations and developing accommodation strategies. Students with disabilities are encouraged to contact the Office for Disability Services at 614-292-3307, room 150 in Pomerene Hall.

Representative schedule:

Lab #	Date	Presentation	Tasks	Assignments
1	Sept 24/25	Safety Review, in vitro rxns Intro to GFP	Streak DH5α-pGLO on 5 plates In vitro rxns, pouring agarose gels	
2	Sept 29/30	PCR & mutagenesis DNA sequence analysis	PCR mutagenesis fluorescence microscopy	Online safety quiz Online prelab quiz
3	Oct 1/2	Controls	Check PCR on gel; *E. coli* transformation	Online prelab quiz Homework #1 due
4	Oct 6/7	Troubleshooting Random vs SDM NEB cutter program	Clean up PCR, digest pGLO/PCRs, set up SDM reaction, pour gels	Online prelab quiz Homework #2 due
5	Oct 8/9		Check digests on gel, set up ligations, *Dpn*I digestion	Online prelab quiz Homework #3 due
6	Oct 13/14		Transform *E. coli*; restreak controls; assemble conj. filter	Online prelab quiz Homework #4 due
7	Oct 15/16	DNA sequencing Translate/Clustal programs	Select mutant and start overnight Set up filter mating	Online prelab quiz
8	Oct 20/21	Plasmid purification Tn mutagenesis Selection vs screen	Mini-preps, submit for DNA sequencing; plate transconjugants	Online prelab quiz Homework #5 due
9	Oct 22/23	Calculate mutation frequency Screening for mutants	Count colonies, identify Lac- mutant, patch colonies for auxotroph/motility	Online prelab quiz Study for quiz
9.5	Oct 23/24		Identify Mot- mutant & restreak	
10	Oct 27/28	Discuss *gfp* seq results Lux fusion, control by lactose	Identify auxotroph, restreak on CAA, measure light from Lac- mutant; glycerol stocks; retest Mot- mutant	Online prelab quiz **QUIZ**
11	Oct 29/30	Genomic DNA; *lac* PCR Genomic sequencing; Aux/Mot assays; BLAST	Genomic DNA preps; set up Lac PCR; submit Aux/Mot for genomic seq; pour agarose gels; set up assays for Aux/Mot	Online prelab quiz Module 1 report due
12	Nov 3/4	Inverse PCR	Lac PCR products on agarose gel; digest genomic DNA of Aux/Mot for iPCR; interpret Aux assays;	Online prelab quiz Homework #6 due
13	Nov 5/6		Submit Lac PCRs for DNA sequence; Dilute ligation step for iPCR; Chemotaxis assays	Online prelab quiz
14	Nov 12/13	Hfr mapping	iPCR – Clean up ligation; *Xmn*I digest Pour agarose gels; interpret chemotaxis	Online prelab quiz
15	Nov 17/18	Hfr mapping	iPCR amplify; agarose gel; submit for DNA sequence; mate Aux/Mot with Hfr strains	Online prelab quiz Module 2 report due
16	Nov 19/20		Pick Hfr strains onto Tet plates	Online prelab quiz
17	Nov 24/25		Count colonies from Hfr crosses	
18	Dec 1/2	Review/repeat		Turn in attendance sheet Module 3 report due
19	Dec 3/4	Final exam		